

Iron transport proteins: Gateways of cellular and systemic iron homeostasis

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Cellular iron homeostasis is maintained by iron and heme transport proteins that work in concert with ferrireductases, ferroxidases, and chaperones to direct the movement of iron into, within, and out of cells. Systemic iron homeostasis is regulated by the liver-derived peptide hormone, hepcidin. The interface between cellular and systemic iron homeostasis is readily observed in the highly dynamic iron handling of four main cell types: duodenal enterocytes, erythrocyte precursors, macrophages, and hepatocytes. This review provides an overview of how these cell types handle iron, highlighting how iron and heme transporters mediate the exchange and distribution of body iron in health and disease.

The year 2017 marks the 20th anniversary of the groundbreaking discovery of DMT1 (divalent metal-ion transporter-1), the first mammalian transmembrane iron transporter to be identified (1). Since then, our knowledge of the molecular mechanisms of iron transport has advanced greatly, as has our understanding of how alterations in the expression/function of iron transport proteins characterize and contribute to iron dyshomeostasis in clinical disorders such as iron-deficiency anemia, the anemia of inflammation, and hereditary hemochromatosis. Iron deficiency is the single most common deficiency in the world and affects an estimated two billion individuals, including 10–20% of young women in the United States (2). The anemia of inflammation (also known as the anemia of chronic disease) is the second most common form of anemia after iron-deficiency anemia (3). Data from the third National Health and Nutrition Examination Survey (1998–1994) indicate that, in the United States, ~700,000 individuals 65 years and older have the anemia of inflammation (4). On the other end of the spectrum of iron disorders is hereditary hemochromatosis, an autosomal recessive iron-overload disease that results predominantly from a C282Y polymorphism in the *HFE* (High Fe) gene (5). The prevalence of this polymorphism is notably high in Caucasian populations: ~1 in 200 to 1 in 300 individuals of northern European descent are homozygous carriers (6). A systematic review of hemochromatosis penetrance concluded that, in the ~1 million C282Y homozygotes in the United

States, up to 38–50% develop iron overload and 10–33% eventually develop hemochromatosis-associated morbidity (7).

Systemic iron homeostasis—from intestinal iron uptake to iron utilization, recycling, and storage—can be largely understood by considering how iron is handled by four main cell types: enterocytes, erythrocyte precursors, macrophages, and hepatocytes. Enterocytes in the proximal small intestine absorb dietary iron; erythrocyte precursors in the bone marrow incorporate iron into heme in red blood cell (RBC) hemoglobin; macrophages in the liver, spleen, and bone marrow recycle iron from senescent RBCs; and hepatocytes store iron (Fig. 1). The aim of this review is to provide a current overview of the proteins that mediate the cellular uptake, intracellular transport, storage, and efflux of iron (as non-heme iron and heme) in these four cell types. Consideration will also be given to how hepcidin, the iron regulatory hormone produced by hepatocytes, regulates systemic iron homeostasis by controlling and coordinating the flow of iron among these various cells.

Dietary iron absorption by the enterocyte

Human diets contain iron as heme or non-heme iron. Heme (iron-protoporphyrin IX) is found in meat, poultry, and seafood and derives mainly from hemoglobin and myoglobin. Non-heme iron refers to various forms of inorganic iron and is usually associated with iron in plants. Foods of animal origin, however, also contain non-heme iron (roughly half of the total iron), mainly in the iron storage protein ferritin. As a consequence, 85–90% of the iron in typical diverse United States diets is non-heme (8). Nonetheless, heme iron is much more bioavailable than non-heme iron, thus making heme an important contributor to iron nutrition. Dietary non-heme iron in the acidic microclimate at the absorptive surface of the proximal small intestine is transported across the apical membrane of enterocytes via DMT1 (SLC11A2, solute carrier family 11 member 2) (Fig. 2A) (1, 9). Mice with intestine-specific deletion of *Slc11a2* display decreased enterocyte iron concentrations, markedly impaired iron absorption (*i.e.* 90% less than control mice), and develop severe iron-deficiency anemia (10, 11). DMT1 therefore appears to be the only mechanism by which non-heme iron is taken up at the intestinal brush border. As a proton-coupled symporter, DMT1 functions optimally at acidic pH by coupling the flow of protons down an electrochemical gradient to the transport of iron into the cell (1). The H⁺ gradient that drives DMT1-mediated iron uptake is generated by intestinal brush-border Na⁺/H⁺ exchanger 3 (NHE3) (12). DMT1 transports only Fe²⁺, but most dietary iron is Fe³⁺ (13); hence, a reduction

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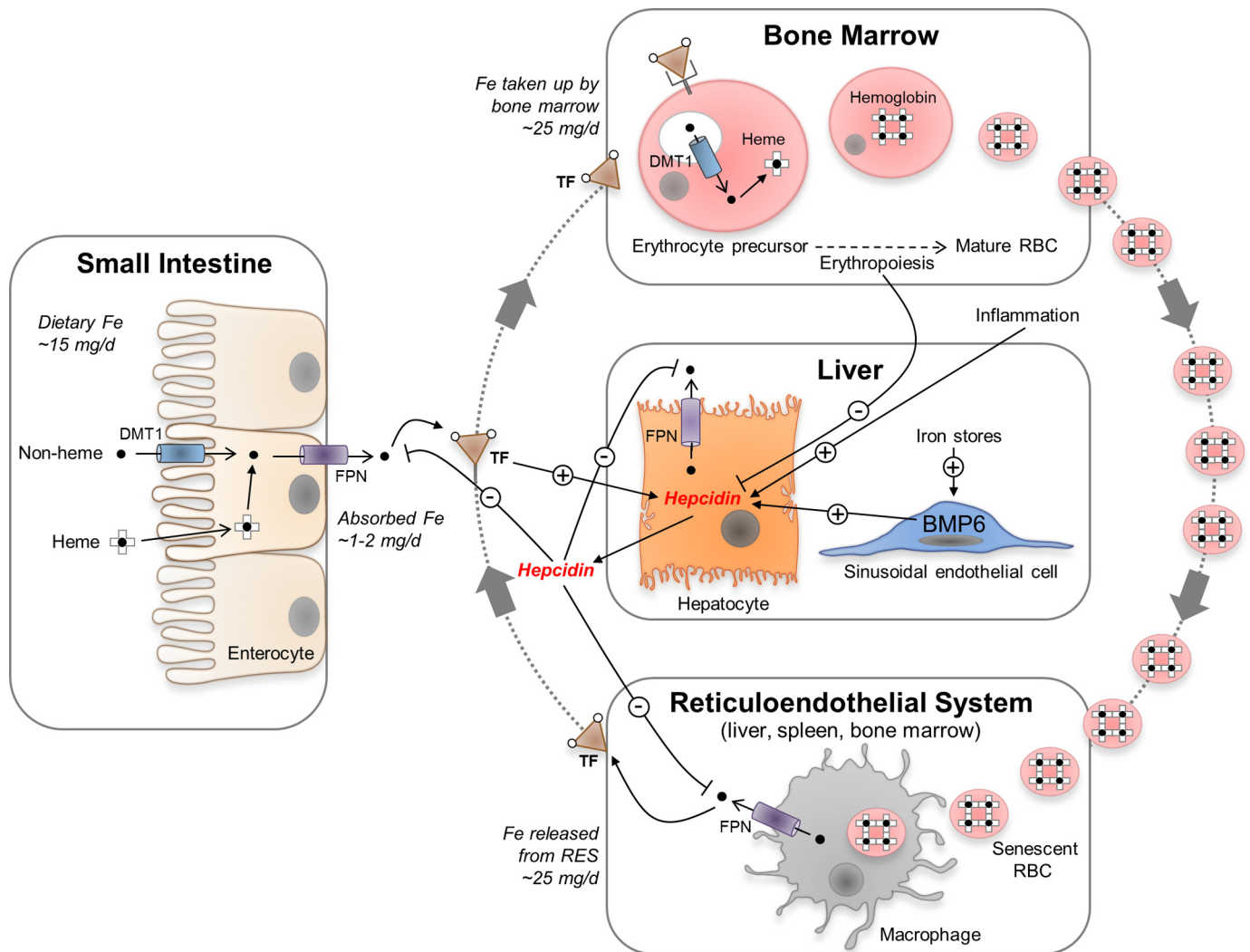


Figure 1. Overview of the main organs and cell types involved in the regulation of systemic iron homeostasis. Enterocytes of the proximal small intestine absorb iron (as Fe^{2+} , ●) into the circulation where it becomes bound (as Fe^{3+} , ○) to its transport protein transferrin. Most circulating iron transferrin is taken up by erythrocyte precursors in the bone marrow, where the iron is incorporated into the heme component of hemoglobin in newly synthesized RBCs. Iron from senescent or damaged RBCs is recycled by macrophages of the liver, spleen, and bone marrow. Hepcidin, the iron-regulatory protein produced by hepatocytes, controls the flow of iron into the plasma by binding to ferroportin (on enterocytes, macrophages, and hepatocytes), causing its internalization and degradation. Hepcidin production is positively regulated by iron transferrin in the plasma, hepatic iron stores, and inflammation. Iron stores are sensed by hepatic sinusoidal endothelial cells, which produce BMP6, a key endogenous regulator of hepcidin expression. Hepcidin synthesis is negatively regulated by increased erythropoietic activity. BMP6, bone morphogenetic protein 6; DMT1, divalent metal-ion transporter-1; FPN, ferroportin; RBC, red blood cell; TF, transferrin.

step is needed before iron transport by DMT1. This activity is generally thought to be mediated by the ferrireductase duodenal cytochrome B (DCYTB²; CYBRD1) located at the apical membrane of enterocytes (14). Consistent with this possibility is the observation that *Dcytb* mRNA expression is markedly up-regulated in iron-deficient mouse intestines (14, 15). *Cybrd1*-null mice, however, show no apparent defects in iron metabolism even in the context of iron deficiency, suggesting that DCYTB can be dispensable for iron absorption (16).

Our understanding of the molecular mechanisms of non-heme iron absorption has advanced primarily through the use of mutant mouse models. Mice, however, are not good models

to study heme iron absorption because they absorb heme very poorly (17). In the 1970s and 1980s, experiments with pig intestine provided evidence that heme is taken up via receptor-mediated endocytosis (18, 19), but little progress has been made since then. It is possible that intestinal heme is taken up via heme-responsive gene-1 (HRG1) protein, a high-affinity heme transporter that transports heme out of the macrophage phagolysosome (20). HRG1 is expressed in human small intestine where it could conceivably function at the apical membrane or in intracellular vesicles (21). Within the enterocyte, heme is degraded by heme oxygenase 1 (HMOX1), and the liberated iron is likely handled in a similar fashion as is absorbed inorganic iron (22).

Iron is transported out of the enterocyte and into portal blood via ferroportin (SLC40A1) located on the basolateral membrane (23–25). Mice with intestine-specific deletion of

² The abbreviations used are: DCYTB, duodenal cytochrome B; PCBP, poly(rC)-binding protein; LRP, low-density lipoprotein receptor-related protein; RES, reticuloendothelial system; TBI, transferrin-bound iron; NTBI, non-transferrin-bound iron; TFR1, transferrin receptor 1; H, heavy.

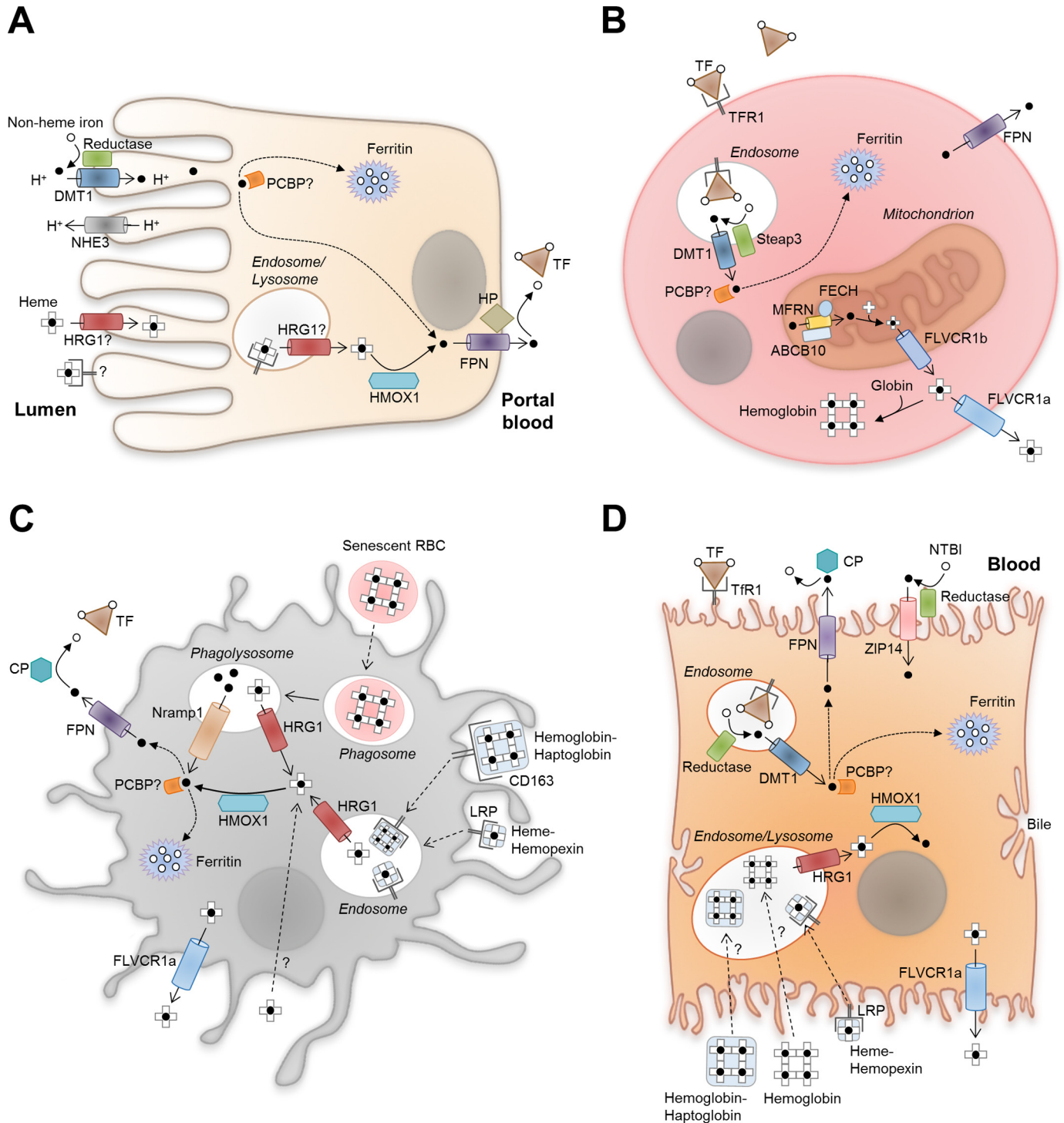


Figure 2. Cellular iron homeostasis. Four cell types are responsible for most of the routine iron handling in the body. *A*, enterocyte; *B*, erythrocyte precursor; *C*, macrophage; and *D*, hepatocyte. Depicted are the main pathways involved in the uptake, intracellular transport, storage, and efflux of non-heme iron and heme in these cells. *ABCB10*, ATP-binding cassette, subfamily B, member 10; *CD163*, cluster of differentiation 163; *CP*, ceruloplasmin; *DMT1*, divalent metal-ion transporter-1; *FECH*, ferrochelatase; *FLVCR1* (feline leukemia virus subgroup C receptor 1); *FPN*, ferroportin; *HMOX1*, heme oxygenase 1; *HP*, hephaestin; *HRG1*, heme-responsive gene-1; *LRP*, low-density lipoprotein receptor-related protein; *MFRN*, mitoferrin 1; *NRAMP1*, natural resistance-associated macrophage protein 1; *NHE3*, Na⁺/H⁺ exchanger 3; *RBC*, red blood cell; *STEAP3*, six-transmembrane epithelial antigen of the prostate; *TF*, transferrin; *TFR1*, transferrin receptor 1; *ZIP14*, ZRT/IRT-like protein-14.

Slc40a1 accumulate iron in duodenal enterocytes and become severely anemic within weeks, demonstrating that ferroportin is essential for intestinal iron export (26). Ferroportin transports only Fe²⁺ (27), whereas transferrin in portal blood will bind only Fe³⁺. Efficient transfer of iron to portal blood trans-

ferrin is thought to involve an oxidation step catalyzed by a ferroxidase. The best characterized intestinal ferroxidase is hephaestin, a membrane-anchored homologue of the plasma ferroxidase, ceruloplasmin. Hephaestin was identified as the gene mutated in the sex-linked anemia (*sla*) mouse, which dis-

plays impaired iron absorption and mild iron-deficiency anemia (28). Accordingly, whole-body as well as intestine-specific hephaestin knock-out mice exhibit iron accumulation in enterocytes and mild iron-deficiency anemia (29). However, the anemia in *sla* mice and hephaestin knock-out mice is only observed in early life, suggesting that hephaestin is dispensable for iron absorption in the adult animal.

How iron that is taken up by DMT1 at the apical membrane traverses the cytosol to reach basolateral ferroportin is unknown, but may involve one or more members of the poly(rC)-binding protein (PCBP) family of metallochaperones. PCBP1 and PCBP2 have been shown to function as iron chaperones that deliver cytosolic iron to ferritin for storage (30, 31). Studies in HEP-2 cells, a human epithelial cell line, have found that PCBP2 (but not PCBP1) physically interacts with DMT1 and ferroportin (32, 33), but whether this occurs in enterocytes or other cell types remains to be determined. Iron directed for storage in ferritin would serve to retain iron in the enterocyte and prevent its absorption into the body. This iron would be eventually lost through the routine sloughing of enterocytes, which turn over approximately every 3 days in humans and mice (34). Ferritin is a large multimeric protein composed of 24 heavy (H) or light subunits in various proportions depending on the tissue. The H subunit has ferroxidase activity that converts Fe^{2+} to Fe^{3+} for storage in ferrihydrite aggregates within the ferritin core (35). Mice with intestine-specific deletion of ferritin H display decreased intestinal iron concentrations and elevated body iron stores, indicating that ferritin H in enterocytes helps to regulate iron absorption (36). Accordingly, up-regulation of enterocyte ferritin resulting from ablation of intestinal iron regulatory proteins decreases iron absorption, even while DMT1 and ferroportin are markedly up-regulated (37).

Iron metabolism in erythrocyte precursors

Greater than 95% of iron in plasma is bound to its circulating transport protein transferrin, which delivers most of its iron to erythrocyte precursors—*i.e.* erythroid progenitor cells of the bone marrow that differentiate into mature RBCs. Each day, ~25 mg of iron is taken up into these cells to support the daily production of 200 billion new RBCs (Fig. 1). Erythrocyte precursors take up iron nearly exclusively from transferrin via transferrin receptor 1 (TFR1) (Fig. 2B). The quantitative importance of this uptake is illustrated by the fact that an estimated 80% of total body cellular TFR1 is located in the erythroid marrow of an adult human (38). When transferrin binds to TFR1 at the cell surface, the complex is internalized into endosomes, which become acidified, causing iron to dissociate from transferrin. The endosomal ferrireductase STEAP3 (six-transmembrane epithelial antigen of the prostate 3) reduces the liberated Fe^{3+} to Fe^{2+} (39), which is subsequently transported into the cytosol via DMT1 (9, 40). Although it is generally accepted that endosomal Fe^{3+} is reduced after it is released from transferrin, thermodynamic and kinetic considerations suggest that reduction occurs (via STEAP3 or otherwise) while Fe^{3+} is still bound to transferrin (41). Interestingly, mice deficient in either STEAP3 or DMT1 (in the hematopoietic compartment) become anemic, but hemoglobin levels can be maintained at ~8 g/dl (10, 39), suggesting that erythrocyte precursors may

have alternative, although less efficient, means of endosomal ferrireduction and iron transport.

In erythrocyte precursors, transferrin-derived iron that enters the cytosol is nearly all directed to mitochondria, where heme is synthesized. Precisely how iron in the cytosol is delivered to the mitochondria is unknown. It is possible that cytosolic iron (bound to low-molecular-weight ligands) freely diffuses into the permeable outer mitochondrial membrane or that it is chaperoned to the mitochondria by PCBPs. Some evidence supports a “kiss and run” mechanism in which endosomal iron is transferred directly from DMT1 into the mitochondria (42). Iron crosses the inner mitochondrial membrane via mitoferrin 1 (SLC25A37) in erythroid cells (43). Non-erythroid cells take up iron into the mitochondria via mitoferrin 1 and its paralogue mitoferrin 2 (SLC25A38) (44). Mitoferrin 1 forms a complex with several proteins, including ferrochelatase and ABCB10 (ATP-binding cassette, subfamily B, member 10), formerly known as ABC-me (45, 46). The association of mitoferrin 1 with ferrochelatase, the terminal enzyme in heme synthesis that inserts an iron in protoporphyrin IX to produce heme, likely serves to couple iron transport into the mitochondria to heme biosynthesis. The interaction between mitoferrin 1 and ABCB10 increases mitoferrin 1 stability thereby increasing iron transport into the mitochondria (47).

Heme appears to be transported out of the mitochondria via isoform b of FLVCR1 (feline leukemia virus subgroup C receptor 1). FLVCR1 was identified as the cell-surface receptor for feline leukemia virus, subgroup C, which infects cats and causes pure red cell aplasia characterized by a block in erythroid differentiation (48). Heme export by FLVCR1 was demonstrated by transport studies using ^{55}Fe -heme and zinc mesoporphyrin (49). The *FLVCR1* gene encodes two protein isoforms as follows: FLVCR1a, a plasma membrane protein, and FLVCR1b, a mitochondrial isoform having an N-terminal mitochondrion-targeting sequence (50). Suppression of *Flvcr1b* expression *in vitro* results in mitochondrial heme accumulation and termination of erythroid differentiation, whereas overexpression of *Flvcr1b* leads to intracellular heme accumulation and erythroid differentiation (50). Evidence for a role *in vivo* is indirectly provided by studies of *Flvcr1*-null mice. *Flvcr1*^{−/−} mice lack both isoforms and die *in utero* due to a block in erythroid differentiation (49), whereas embryos lacking only the 1a isoform have normal erythropoiesis (50), suggesting that Flvcr1b activity is sufficient for erythropoiesis, at least at the fetal stage. Studies of *Flvcr1b*-deficient mice are needed to clarify the *in vivo* role(s) of the 1b isoform.

Despite being the most avid consumers of iron in the body, erythrocyte precursors abundantly express ferroportin at the plasma membrane (51) and are therefore able to export non-heme iron. Systemic iron depletion has been shown to increase ferroportin expression in erythrocyte precursors, leading to the hypothesis that up-regulation of ferroportin in iron deficiency serves to provide iron to non-erythropoietic tissues (52).

Macrophage iron metabolism

Senescent or damaged RBCs are cleared from the circulation by macrophages of the liver, spleen, and bone marrow, which are collectively referred to as the reticuloendothelial system

(RES) (53). Each day, RES macrophages reclaim and recycle ~25 mg of iron from RBCs, nearly all of which is returned to the bone marrow for reincorporation into newly synthesized heme in erythrocyte precursors (Fig. 1). By comparison, only about 1–2 mg of iron is absorbed each day by enterocytes, indicating that the vast majority of iron used for RBC synthesis is from iron recycled by the RES.

During erythrophagocytosis, macrophages engulf and internalize RBCs into the phagosome, which fuses with lysosomes to become a phagolysosome (Fig. 2C). Hydrolytic enzymes within the phagolysosome degrade the RBC and its hemoglobin to release heme, which is translocated across the phagolysosomal membrane into the cytosol via HRG1 (20). In RES macrophages, HRG1 specifically localizes to the erythrophagolysosome and is up-regulated by erythrophagocytosis (20). Cytosolic heme induces the expression of HMOX1, a cytoplasm-facing endoplasmic reticulum-anchored enzyme that degrades heme into iron, CO, and biliverdin (54). Given that each mature RBC has $\sim 1.2 \times 10^9$ heme moieties, and that free heme is highly cytotoxic, very efficient mechanisms must exist for dealing with such a large bolus of heme following erythrophagocytosis (55). HMOX1 is one essential mechanism, as illustrated by the fact that *Hmox1*-deficient macrophages do not survive erythrophagocytosis *in vitro* (56). Accordingly, *Hmox1*-null mice show a progressive and nearly complete loss of macrophages in the liver and spleen by 12 months of age (56). Heme export via FLVCR1a, which has been demonstrated in bone marrow-derived macrophages, may serve as another mechanism to protect the cell from heme toxicity (49).

It has been proposed that some heme may be degraded within the phagolysosome to release iron that is transported into the cytosol via NRAMP1 (natural resistance-associated macrophage protein 1), a DMT1 homologue located on phagolysosomal membranes (57, 58). Consistent with this model is the observation that *Nramp1*^{-/-} mice show impairments in erythrocyte recycling after hemolytic stress (59). Whatever the function of NRAMP1 in macrophage iron recycling, the effect appears to be small because *Nramp1*^{-/-} mice have normal hematocrits and only slightly elevated concentrations of splenic non-heme iron (59).

Macrophages can additionally take up hemoglobin and heme from the circulation. Hemoglobin appears in blood plasma mainly from intravascular hemolysis, a physiologic process that accounts for at least 10% of red blood cell breakdown in normal individuals (60). Hemoglobin in the plasma binds to the acute-phase protein haptoglobin to form a hemoglobin-haptoglobin complex that is recognized by the scavenger receptor CD163, which is exclusively expressed on macrophages (61, 62). Hemoglobin that is not bound to haptoglobin can also be taken up by CD163 (63). Interestingly, hemoglobin clearance from the plasma is largely unaffected in mice lacking CD163 (64), indicating that alternative hemoglobin and hemoglobin-haptoglobin clearance pathways exist (such as in hepatocytes as noted below). Extensive hemolysis can deplete plasma haptoglobin, giving rise to free hemoglobin. When this occurs, ferrous (Fe²⁺) hemoglobin becomes oxidized to ferric (Fe³⁺) hemoglobin, resulting in the release of heme, which binds to the plasma glycoprotein hemopexin. Hemopexin sequesters heme in a

non-reactive form and transports it to the liver, where it is taken up by Kupffer cells via LRP (low-density lipoprotein receptor-related protein, also known as CD91) (65). After endocytosis, heme is released from hemopexin and transported into the cytosol, where it is catabolized by HMOX1. Interestingly, studies of hemopexin-null mice reveal that that Kupffer cells can also acquire heme independently of hemopexin (66).

Hepatocyte iron metabolism

Hepatocytes take up iron in a variety of forms: transferrin-bound iron (TBI), non-transferrin-bound iron (NTBI), hemoglobin, and heme (Fig. 2D). TBI is taken up by receptor-mediated endocytosis after it binds to transferrin-receptor 1 (TFR1) located at the sinusoidal membrane (67). Acidification of endosomes causes transferrin to release its Fe³⁺, which is subsequently reduced to Fe²⁺ and then transported into the cytosol via DMT1. DMT1 plays a role in this process that is demonstrated by studies showing that mice with hepatocyte-specific inactivation of DMT1 take up to 40% less iron from TBI when compared with wild-type controls (68). Interestingly, mice lacking DMT1 in hepatocytes have normal hepatic iron concentrations and display no impairments in loading, indicating that hepatocyte DMT1 is dispensable for the overall iron economy of the liver. Indeed, although hepatocytes are capable of taking up TBI, this pathway is likely to be minimal under normal circumstances because hepatocytes are a primary site of iron storage, and iron-replete conditions down-regulate TFR1 expression. Perfusion studies have shown that only 3% of iron bound to transferrin is taken up by normal rat liver (69).

It is well known that the liver rapidly takes up NTBI, which appears in the plasma of patients with iron overload (70). NTBI refers to a heterogeneous mixture of low-molecular-weight forms of iron that become detectable in plasma when transferrin saturations exceed 75% (71). NTBI is taken up by the liver via ZRT/IRT-like protein-14 (ZIP14/SLC39A14), a transmembrane metal-ion transporter located on the sinusoidal membrane of hepatocytes (72). ZIP14 was originally identified as a zinc transporter, but subsequent studies showed that it could also transport iron (73, 74) and that its protein levels in liver increase in response to iron loading (72). *Slc39a14*-null mice display markedly impaired uptake of intravenously administered ⁵⁹Fe-NTBI and fail to load iron in hepatocytes when the mice are fed an iron-loaded diet or crossed with mouse models of hereditary hemochromatosis (75). Therefore, ZIP14 is the major, and perhaps only, NTBI uptake mechanism in hepatocytes. Although the exact chemical nature of NTBI is unknown, most NTBI appears to be ferric species, with one study identifying ferric citrate as the major form in plasma from patients with hemochromatosis (76). Given that ZIP14 transports Fe²⁺ but not Fe³⁺, a reduction step, likely involving a reductase (77), is needed for ZIP14-mediated uptake of plasma NTBI. Part of this reductase activity may be provided by prion protein (PrP^c), as *PrP*^{-/-} mice have been shown to take up 30% less ⁵⁹Fe-NTBI into the liver than do control mice (78). Moreover, functional cooperation between PrP^c and ZIP14 is suggested by the observation that co-expression of both proteins in hepatoma cells resulted in greater cellular iron uptake than when either protein was expressed alone (79).

Like macrophages, hepatocytes can also acquire iron in the form of hemoglobin and heme. Isolated rat hepatocytes take up not only hemoglobin-haptoglobin but also free hemoglobin (80), which may help to explain why haptoglobin knock-out mice show no defects in the clearance of free hemoglobin from the circulation (81). Heme bound to hemopexin is taken up via LRP expressed by hepatocytes (65). Hemopexin seems to be required for this process because hemopexin-null mice show minimal heme loading in hepatocytes after heme overload (66). The observation that hemopexin-null mice, as well as haptoglobin-null mice, have normal hepatic non-heme iron levels suggests that heme iron uptake pathways are not significant contributors to hepatic iron stores under normal circumstances (82, 83).

Iron is released from hepatocytes via ferroportin located on the sinusoidal membrane. Mice with hepatocyte-specific deletion of ferroportin exhibit impaired hepatic iron mobilization and anemia in response to phlebotomy or an iron-deficient diet (84). Iron mobilization from hepatocytes additionally requires ceruloplasmin, a circulating ferroxidase homologous to the intestinal ferroxidase hephaestin (85). As with hephaestin, ceruloplasmin oxidizes Fe^{2+} to Fe^{3+} for loading onto apotransferrin. Ceruloplasmin knock-out mice exhibit hepatocyte iron loading (85), which may arise because ferroxidase activity is required for iron efflux from the liver (86).

Although hepatocytes function in the scavenging and catabolism of excess heme, it is interesting to note that hepatocytes export heme, as hepatocyte-specific deletion of *Flvcr1a* in mice causes heme to accumulate in hepatocytes, despite an induction of HMOX1 (87). The accumulation is associated with heme biosynthesis, suggesting that FLVCR1a exports newly synthesized heme that is not degraded or utilized by the cell.

Hepcidin, master regulator of systemic iron homeostasis in health and disease

In addition to their role as an iron-storage cell, hepatocytes help to regulate systemic iron homeostasis by producing hepcidin, a 25-amino acid peptide hormone (88, 89). Circulating hepcidin controls the amount of iron released into the blood plasma by inhibiting iron export from macrophages, enterocytes, and hepatocytes (Fig. 1) (90–92). At the molecular level, hepcidin inhibits iron export by binding to cell-surface ferroportin and initiating its internalization and degradation in lysosomes (93). Hepcidin expression is suppressed when the rate of erythropoiesis increases (e.g. in anemia) and is increased by plasma iron (iron transferrin), iron stores, and inflammation (94). In iron-deficiency anemia, the body seeks to return to iron balance by increasing the absorption of dietary iron. This is achieved by down-regulating the expression of hepcidin, which increases ferroportin expression in enterocytes thereby increasing iron transfer into portal blood (Fig. 2A). Alterations in hepcidin expression also account for the iron overload in hereditary hemochromatosis. Most hemochromatosis arises from an inability to produce sufficient amounts of hepcidin (95), resulting in increased iron absorption that, over time, leads to iron overload because the body has no active mechanisms to rid itself of excess iron. Furthermore, low hepcidin levels in hemochromatosis increase ferroportin expression in

RES macrophages. Increased iron efflux from macrophages raises plasma iron concentrations to levels that saturate the binding capacity of transferrin, giving rise to NTBI, which is taken up into hepatocytes via ZIP14 (Fig. 2D) (75). Indeed, these systemic and cellular alterations readily explain the hallmark clinical features of hereditary hemochromatosis: elevated transferrin saturation and hepatic iron overload, with iron accumulating in hepatocytes and not in macrophages (96). Under normal circumstances, elevations in hepatic iron stores are accompanied by an increase in hepcidin expression, which down-regulates intestinal ferroportin expression and iron absorption. The amount of iron stores is somehow sensed by sinusoidal endothelial cells, which produce BMP6 (bone morphogenetic protein 6) (97), a positive regulator of hepcidin expression (Fig. 1) (98). Hepcidin expression is also positively regulated by acute increases in transferrin saturation, such as after a large-dose oral iron supplement. The increase results from a direct interaction between iron transferrin and hepatocytes (Fig. 1) (99). Hepcidin expression is independently up-regulated by inflammation, which helps to explain some of the clinical features observed in the anemia of inflammation, namely elevated plasma hepcidin levels decrease the expression of ferroportin in macrophages, leading to macrophage iron retention and hypoferremia. Although elevated plasma hepcidin levels in inflammation would also decrease intestinal ferroportin expression and iron absorption, the contribution of iron absorption to plasma iron is small ($\sim 1\text{--}2$ mg/day) compared with that of macrophages (~ 25 mg/day) (Fig. 1). The regulation of hepcidin expression has been studied extensively and is well covered in recent reviews (94, 100).

Conclusions and future directions

In the past 20 years, the study of iron biology has been transformed and revitalized by the discovery of mammalian iron transport proteins (e.g. DMT1, ferroportin, mitoferrin 1, and ZIP14), heme transporters (e.g. HRG1 and FLVCR1), iron chaperones (i.e. PCBP_s), ferrireductases and ferroxidases (e.g. DCYTB, STEAP3, PrP^c, and hephaestin), and the iron-regulatory hormone hepcidin. Elucidation of the physiologic function of these proteins has been achieved predominantly through the generation of global and, in many cases, conditional knock-out mouse models. What will the next 20 years bring? Research into the less well-understood organs/cell types is certain and will likely reveal novel iron transporters, related proteins, and their regulators. Indeed, many fundamental questions remain. For example, how is iron transported across the blood-brain barrier? This is important because brain iron deficiency in early life may lead to irreversible cognitive deficits, whereas too much brain iron can lead to neurodegeneration. How is iron handled by the kidney? Very little iron is lost in the urine, even in iron overload, indicating that the kidney has efficient mechanisms for reabsorbing iron filtered by the glomerulus. How does iron cross the placenta? During pregnancy in humans, about 300 mg of iron is transferred from mother to fetus across the placenta, but how this occurs remains a mystery. Answers to these and other important research questions in the years to come will continue to shape and refine our ever-changing picture of iron transport and its role in health and disease.

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